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Variations in Leaf Photosynthetic and Morphological Traits with Tree Height in Various Tree Species in a Cambodian Tropical Dry Evergreen Forest

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Abstract

In this study, we demonstrate changes in leaf morphological and physiological traits with tree height from dark understory to bright canopy conditions in various tree species in the Cambodian tropical dry evergreen forest. The vegetation mainly consisted of Dipterocarpaceae and Myristicaceae and the canopy trees usually reached 30-40 m in height. We investigated 25 individuals of 18 tree species ranging from 0.8 to 33 m in height. We measured the leaf photosynthetic rate, stomatal conductance and respiration rate for 3 to 5 leaves per sampling position in the early dry season. All leaves were then divided into two parts: one for measuring dry weight, nitrogen content and $\delta^{13}C$; the other for observation of leaf morphology. The leaf morphological traits, such as leaf mass per area (LMA), cuticle thickness, palisade layer thickness, leaf hardness and stomatal density increased linearly with tree height. The leaf nitrogen content per unit leaf area (N_{area}) peaked at 10 m from the ground, though the nitrogen content per unit dry leaf mass (N_{mass}) decreased linearly with tree height. Higher LMA, cuticle thickness and hard leaves in canopy condition may contribute to high drought tolerance and physical strength. The leaf-area-based photosynthetic rate ($A_{max\text{-}area}$) peaked at an intermediate tree height of approximately 10 m, and then decreased toward the upper canopy. In contrast, the leaf-mass-based photosynthetic rate ($A_{max\text{-}mass}$) decreased linearly with tree height. Reduction of leaf nitrogen content and stomatal conductance mainly limit photosynthetic capacities with tree height. Overall, many leaf morphological traits could be summarized in a simple and significant relation with tree height, though increasing tree height, which is related to the micro-climatic gradient, leads to both nitrogen and stomatal constraints of leaf photosynthetic capacities, even when considering many different tree species.

Discipline: Forestry and forest products
Additional key words: Cambodia, LMA, photosynthesis, respiration, stomatal conductance

Introduction

The tropical dry forest in the monsoon area of Southeast Asia has deteriorated rapidly due to land conversion, forest fragmentation, and degradation by disturbances resulting from the expansion of human activities in agriculture and timber-logging[9, 16]. In particular, the decline of forest area in Cambodia, which still maintains relatively high forest coverage (59.1% in 2006) in this region, has

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accelerated in the recent decade[16]. The forest was lost at an average annual rate of 2.1% from 2002 to 2006, which is the swiftest rate of deforestation among neighboring countries[16, 20]. Recent research revealed that those tropical dry forests are crucial for CO_2 storage and annual primary production in the region[7, 39, 62, 68]. However little is known about the leaf photosynthetic trait, which is essential for accurate estimation and/or modeling of those forest functions, in the various trees of the Cambodian tropical dry forest.

Tropical dry forests, especially evergreen and semi-evergreen, include complex and multilayered vertical structures, meaning the forest environment varies significantly with the forest height[25, 50]. The crown surface of the canopy trees in the forest is usually exposed to strong sunlight, whereas less than 2% of solar radiation typically reaches the forest floor[71]. The tree leaves in the canopy also experience strong desiccating conditions due to the higher irradiance, vapor pressure difference (VPD), temperature, hydraulic constraint and stronger wind than leaves in the understory[8, 37, 40, 56, 57, 70]. In contrast, the forest understory is usually dark and humid with high CO_2 concentration. How do tree leaves respond to the drastic environmental differences at differing heights in the forest? Understanding of the changes in leaf physiological and other characteristics, especially in terms of photosynthetic capacities based on forest height facilitates CO_2 fixation model in the forest[13, 43]. Many tree species have photosynthetic capacities that differ according to their growth stages and/or light conditions, through changes in their leaf morphological and biochemical properties[44]. It is well known that leaves under bright conditions (sun leaves) have greater leaf nitrogen and leaf mass per area (LMA), corresponding to higher photosynthetic capacities, than those in dark conditions (shade leaves). Shade leaves have a higher leaf chlorophyll content and are thinner, in order to maintain a dark respiration rate and light compensation point which are lower than in sun leaves[41, 44].

Photosynthetic capacities in canopy trees of the tropical dry forest may be limited with tree height, even if their leaves receive sufficient sunlight, though several tropical rainforest trees have shown a strong linear increment of leaf photosynthetic capacities with tree height[31, 55]. This is because environmental stresses such as temperature, strong sunlight and drought stress are usually severe in the canopy of a tropical dry forest compared with a rainforest. In fact, a recent study of the growth of many tall tree species in a temperate forest, with seasonal changes in rainfall and temperature, revealed that even in canopy leaves under bright conditions, their photosynthetic capacities were limited by a stressful canopy environment[28, 40, 48, 66, 67]. However, we have little information on the vertical variations in leaf photosynthetic, morphological and biochemical traits with tree height in tropical dry forests in Southeast Asia. In particular, studies on these traits in Cambodian tropical forests are quite scarce, except for a preliminary report by Hozumi et al. (1969a).

In this study, our objective is to demonstrate the vertical changes on photosynthesis-related leaf physiology and morphology in various species of Cambodian tropical trees. For this purpose, we measured essential parameters to understand the forest carbon exchange mechanisms, such as the leaf maximum photosynthetic rate, dark respiration rate, stomatal conductance, nitrogen content, and LMA under conditions ranging from dark understory to bright canopy. We also expected that these leaf traits, especially the maximum photosynthetic rate, would change with tree height and be inter-related in the Cambodian dry tropical forest.

Materials and methods

1. Study site

The study was carried out in Kampong Thom, Cambodia (12°44’N, 105°28’E). Annual rainfall and mean temperature were 1540 mm and 27°C, respectively. Most rainfall (approx. 90%) occurred during the rainy season from May to October. The vegetation on the study site was classified as dry evergreen forest, which accounted for more than 34% of the total forest land of Cambodia in 2006[20]. Most of the canopy trees were 30–40 m high; mainly consisting of Dipterocarpaceae, Myristicaceae and Clusiaceae[49]. The soil type is mainly Acrisols based on FAO and WRB classification[68].

All measurements were conducted in November to December 2009, which is the beginning of the dry season. In the early dry season, leaf photosynthetic activities seemed high, especially in the dry evergreen forest, with high water storage in a thick soil layer[27, 61, 62]. In fact, the stomatal conductance in several trees showed relatively higher value in the early dry season[10] and soil and regolith were widely distributed throughout the study site[51] to a thickness exceeding 10 m.

2. Measurement of relative photosynthetic active radiation and temperature with height gradient

The relative photosynthetic active radiation (PAR, %) to an open condition at 60 m in height and temperature (°C) were measured at different height gradients from a meteorological observation tower. We estimated relative PAR by the simple recording film method[36]. Three films (Opt-leaf R3D, Taisei E & L Inc, Japan) were set horizontally for each measurement point at 1, 2, 5, 10, 15, 20, 25, 30, 35, 40 and 60 m from the ground, respectively. All films were exposed for 3 days, including a day to measure the leaf physiology, and the discoloring rate was recorded. The period was almost exclusively sunny. The value at the 60 m was taken as the open condition. The spectral sensitivity of the film peaked at 521 nm[36], meaning it did not correspond exactly with that of the quantum sensor (approx. 400-700 nm). To reduce possible error resulting from these differences, the regression, which was obtained from various forest positions such as gap and understory, between the discoloring rate (DR) and the integrate PAR measured using the quantum sensor (IKS-27, Koito, Japan) was used in this study[36].

$$\mathrm{PAR} = -103.6\mathrm{DR}^2 + 107.16\mathrm{DR} + 200.53$$

The air temperature at 2 and 40 m was also measured at 10 minute intervals using a thermo recorder (TR-51, T&D Corporation, Japan).

3. Plant materials and sample collection

We selected 25 individuals of 18 tree species ranging from 0.8 to 33 m in height for the study (Appendix 1). The species included typical late successional and gap species such as *Dipterocarpus*, *Anisoptera*, *Calophyllum*, *Myristica*, *Ficus*, *Macaranga* and *Anthocephalus* in the study area. We collected 1 - 3 branches per sampling position by using a 60 m-high meteorological observation tower[60]. The sampled shoots were immediately placed in water to prevent the stomata closing, hence our measuring values on photosynthesis were considered potential values, because the cut shoots were released from hydraulic constraints such as path length or gravity on leaf water potential after cut treatment[1]. Several studies showed that cut shoots represented intrinsic leaf photosynthetic properties in the absence of the direct influence of this gravity on leaf water potential[1, 28, 69]. Our preliminary experiment also showed that leaves at detached shoots retained similar photosynthetic rates and/or stomatal conductance compared with the attached condition at least 10 to 20 minutes after cutting. *Dipterocarpus alatus*, *Anisoptera costata*, *Ficus* sp., *Myristica iners* and *Macaranga griffithiana* were all used for the preliminary experiment. We also directly measured the leaf photosynthetic rate at the attached condition for sampled trees lower than 3 m in height.

4. Measurement of leaf physiological and morphological characteristics

Leaf ecophysiological traits related to photosynthesis were measured using a portable photosynthesis meter (LI-6400, Li-Cor, Lincoln, NE). We also measured the leaf gas exchange rate from 2 to 5 leaves per sampled branch. All measurements were conducted before 12:00 to avoid the midday depression of photosynthesis [10, 29]. The relation between the photon flux density and carbon assimilation rate was determined for fully expanded leaves and apparently non-senescing leaves. The measuring light intensities were 0, 50, 100, and 1500 (µmol photon m^{-2} s^{-1}) and the temperature was approximately 30°C. The CO_2 concentration and air humidity in the leaf chamber were maintained at 360 ppm and approximately 60%, respectively. The photosynthetic rate at light saturation ($A_{\text{max-area}}$, µmol m^{-2} s^{-1}) and the dark respiration rate (R_d, µmol m^{-2} s^{-1}) were obtained from the measurements[33]. The maximum stomatal conductance ($gs_{\text{max-area}}$, mmol m^{-2} s^{-1}) and transpiration rate (*Tr*, mmol m^{-2} s^{-1}) at light saturation were also recorded and water use efficiency (*WUE*), which is the ratio of $A_{\text{max-area}}$ and $gs_{\text{max-area}}$, was calculated[33, 34].

The sampled leaves were then divided into two parts without large veins: one for measuring leaf hardness, leaf mass per area (LMA, g m^{-2}), nitrogen content and stable carbon isotope composition ($\delta^{13}C$, ‰); the other for observing leaf morphology such as stomatal density and the mesophyll structure[30]. Leaf hardness (N mm^{-2}) was measured by using a penetrometer (Aikoh Engineering Co., Tokyo, Japan) with a straight metal rod 2 mm in diameter[35]. This method measures the maximum load required for the metal rod to penetrate a leaf blade[35, 52]. Leaf nitrogen and carbon content were also measured by an NC analyzer (FLASH EA112, Thermo Science, Waltham, MA, USA) after all leaves had been dried at 60°C for 3 days. The stable carbon isotope composition of the leaf samples was determined using an isotope ratio mass spectrometer (Delta Plus System; Thermo Quest, San Jose, CA, USA). The overall carbon isotope ratio was expressed in delta notation relative to the PD Belemnite standard: $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$ (‰), where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample, and $R_{standard}$ is the ratio of the standard[63]. The leaf morphological structures, such as the thicknesses of the cuticle and palisade layer and leaf epidermis were determined with a micrometer under a micrograph at 200x magnification[30]. To determine stomata density, we made an impression of the under surfaces of the leaves using quick-drying adhesive. These replicas were observed under an optical microscope, and the density of stomata was calculated.

Results and discussion

1. Changes in light and temperature with tree height

The canopy leaves were exposed to high environmental stress such as higher temperature and light intensity compared with understory leaves and such conditions largely varied with forest height in the Cambodian tropical dry forest (Figs. 1, 2). The maximum temperature at the canopy condition (40 m from the ground) was

Appendix 1. Studied tree species, successional status (SS), sampling height (H, m), leaf-area-based photosynthetic rate ($A_{max\text{-}area}$, μmol m^{-2} s^{-1}), stomatal conductance ($gs_{max\text{-}area}$, mmol m^{-2} s^{-1}), transpiration rate ($Tr_{max\text{-}area}$, mmol m^{-2} s^{-1}), dark respiration rate (R_d, μmol m^{-2} s^{-1}), leaf mass per area (LMA, g m^{-2}) and nitrogen content (N_{area}, g m^{-2})

Family	Species	SS	H	$A_{max\text{-}area}$	$gs_{max\text{-}area}$	Tr_{area}	R_d	LMA	N_{area}
Cluciaceae	*Calophyllum calaba*	LS	30	3.22	0.09	3.92	-2.09	126	1.88
	Calophyllum calaba	LS	30	6.46	0.18	6.73	-2.25	116	1.46
	Calophyllum calaba	LS	2	3.97	0.06	2.48	-0.54	90	0.95
	Calophyllum calaba	LS	2	7.70	0.11	4.00	-0.88	78	0.86
	Calophyllum calaba	LS	1	6.74	0.10	4.62	-0.77	71	1.08
Dipterocarpaceae	*Anisoptera costata*	LS	7.2	6.25	0.06	2.66	-1.13	83	2.26
	Anisoptera costata	LS	3	8.01	0.20	7.64	-2.12	86	1.65
	Dipterocarpus aratus	LS	2	10.08	0.21	6.76	-1.35	48	1.24
	Dipterocarpus aratus	LS	0.8	6.00	0.12	4.41	-0.84	42	0.97
	Dipterocarpus costatus	LS	14	5.82	0.19	5.99	-2.08	119	1.87
	Dipterocarpus costatus	LS	14	5.77	0.19	6.11	-2.15	128	1.96
	Dipterocarpus costatus	LS	14	6.67	0.19	6.41	-1.50	116	2.09
	Dipterocarpus costatus	LS	19.4	7.92	0.20	6.57	-2.19	134	1.84
	Dipterocarpus costatus	LS	19.4	7.80	0.23	7.21	-1.82	149	2.16
	Dipterocarpus costatus	LS	19.4	6.82	0.19	6.55	-1.30	123	1.57
	Dipterocarpus costatus	LS	22.1	6.42	0.11	4.56	-1.54	121	1.76
	Dipterocarpus costatus	LS	22.1	8.31	0.21	7.16	-1.28	123	2.01
	Dipterocarpus costatus	LS	23.5	5.49	0.11	4.42	-1.65	118	1.90
	Dipterocarpus costatus	LS	23.5	5.72	0.11	4.57	-1.69	111	1.74
	Dipterocarpus costatus	LS	23.5	6.23	0.09	4.15	-1.56	134	2.00
	Hopea odorata	LS	4.8	13.42	0.37	8.67	-2.21	96	1.85
Ebenaceae	*Diospyros* sp.	LS	2.5	7.52	0.28	7.71	-2.14	74	1.07
	Diospyros sp.	LS	2.5	5.43	0.09	3.32	-1.81	78	1.07
Elaeocarpaceae	*Elaeocarpus* sp.	LS	7	-	-	-	-	89	1.38
	Elaeocarpus sp.	LS	7	-	-	-	-	86	1.27
Euphorbiaceae	*Macaranga griffithiana*	G	5	12.72	0.22	7.07	-1.22	71	1.45
	Macaranga griffithiana	G	5	10.50	0.13	4.90	-1.36	65	1.47
Loganiaceae	*Fagraea fragrans*	LS	1.5	6.24	0.26	6.94	-1.10	52	0.92
	Fagraea racemosa	G	1	5.05	0.12	3.96	-0.94	60	0.84
Lauraceae	*Litsea* sp.	LS	1	6.11	0.11	3.15	-0.57	76	1.25
Melastomataceae	*Melastoma sp*	G	1	5.98	0.27	8.38	-1.02	45	0.67
	Melastoma sp	G	1	4.96	0.15	5.55	-1.05	47	0.74
Moraceae	*Ficus sp*	G	1	6.76	0.13	4.99	-1.46	31	0.66
Myristicaceae	*Myristica iners*	LS	33	6.66	0.10	4.67	-1.98	146	2.26
	Myristica iners	LS	33	3.71	0.06	2.67	-1.11	149	2.20
	Myristica iners	LS	20	7.46	0.14	5.46	-0.65	122	2.15
	Myristica iners	LS	10	5.77	0.05	1.50	-0.97	110	1.99
	Myristica iners	LS	1	4.00	0.10	2.77	-0.38	45	0.98
Myrsinaceae	*Ardisia* sp.	LS	1.2	5.40	0.19	5.25	-0.77	57	0.87
	Syzygium sp.	LS	16	6.92	0.14	5.54	-1.01	152	2.33
	Syzygium sp.	LS	16	8.07	0.14	5.50	-0.97	151	2.20
	Syzygium sp.	LS	6.5	9.16	0.46	10.30	-1.07	121	1.64
	Tristaniopsis burmanica	LS	29	5.98	0.10	4.56	-1.65	208	1.66
	Tristaniopsis burmanica	LS	20	5.01	0.07	3.01	-0.67	183	1.63
	Tristaniopsis burmanica	LS	20	5.21	0.08	3.57	-0.99	161	1.73

Appendix 1. Continued.

Family	Species	SS	H	$A_{\text{max-area}}$	$gs_{\text{max-areaa}}$	Tr_{area}	R_d	LMA	N_{area}
Rubiaceae	*Anthocephalus chinensis*	G	13.5	9.44	0.13	5.38	-0.55	104	1.74
	Anthocephalus chinensis	G	13.5	10.90	0.15	5.86	-1.91	105	1.90

All data are average value for each branch (3 to 5 leaves). The successional status (SS) was determined in the late successional (LS) and gap (G) species by observation and a literature survey[21].

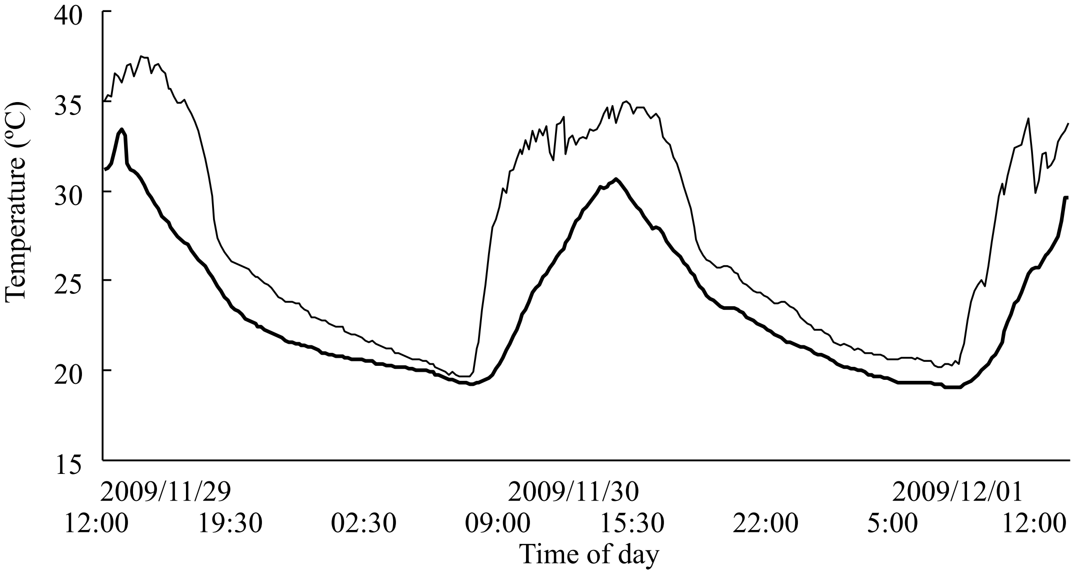

Fig. 2. Diurnal changes in temperature between the canopy (40 m) and understory (2m)
——— : 40 m, ——— : 2 m.

approximately 5°C higher than the understory, reaching 35°C (Fig. 2). The relative PAR also increased significantly with height, from 1.3% at the understory to 90% at 20 m from the ground, though the light condition was virtually constant above 20 m (Fig. 1). This continual change in PAR with height may relate to the continuous dense leaf layer from the understory to a height of approximately 20 to 30 m in this forest. The relative light intensity in neighboring seasonal tropical forest in northeast Thailand also changed significantly with forest height, from less than 2% at the understory to 80% at 30 m[71].

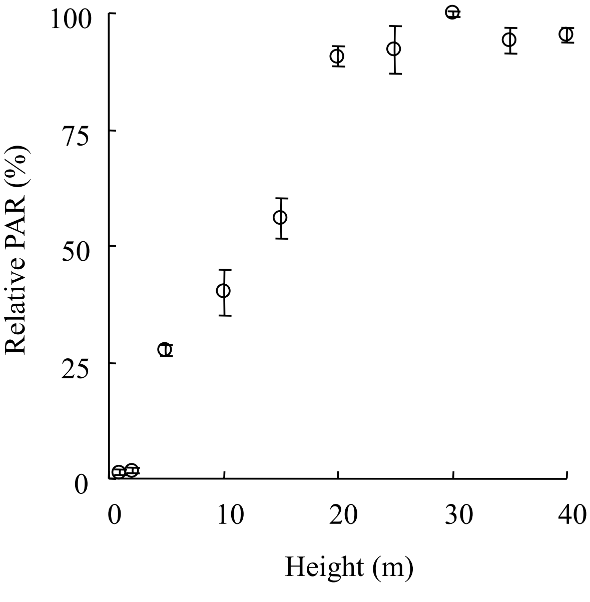

Fig. 1. Relation between height and relative photon flux density (PAR) to open at 60 m in height
Bars indicate the standard error (n = 3 for each position).

2. Changes in leaf morphological traits with tree height

Most leaf morphological traits, such as leaf mass per area (LMA), cuticle thickness, and palisade layer thickness linearly increased with tree height (Fig. 3), even including various tree species with different successional states. These linear changes in leaf traits with tree height may be common in tropical forests in Southeast Asia and

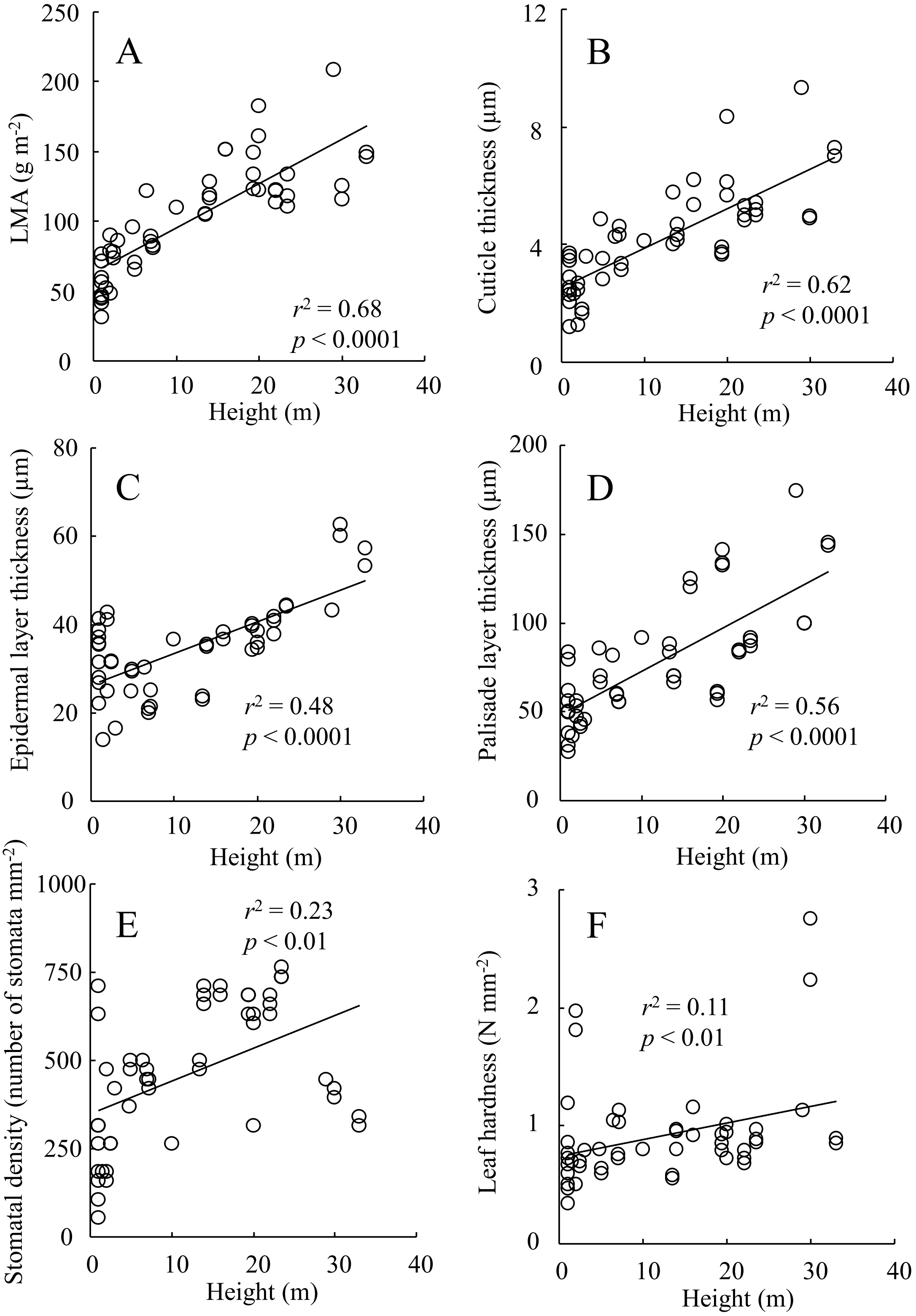

Fig. 3. Relations between the tree height and: leaf mass per area (LMA; A), cuticle thickness (B), leaf abaxial and adaxial epidermis layer thickness (C), palisade layer thickness (D), stomatal density (E) and leaf hardness (F)

The regression lines are: A, $y = 63.3 + 3.2x$. B, $y = 2.56 + 0.13x$. C, $y = 26.34 + 0.72x$. D, $y = 48.14 + 2.45x$. E, $y = 349.7 + 9.3x$, F, $y = 0.738 + 0.014x$.

South America[6, 31, 55].

Higher LMA, cuticle thickness, epidermis thickness, stomatal density and hard leaves in canopy trees may contribute to high drought tolerance and physical strength under the stressful conditions of the canopy environment. In general, many tree species facing stressful environments such as drought and strong sunlight tend to have thicker and larger LMA leaves with high stomatal density in the same tree species[4, 32, 41, 58, 59]. Our results showed that various Cambodian tree species also have similar leaf morphological adaptation to the upper canopy environments with high drought stress. In particular, the thicker cuticle and epidermis cell layer in upper canopy trees help prevent water loss from the leaf surface[14, 35, 53]. Kenzo et al. (2007) also reported that several dipterocarp tree species with higher LMA had high desiccant tolerance under drought conditions in a degraded tropical rainforest. In addition, we found a significant positive relation between the C/N ratio and tree height (Fig. 5C). This change may mean that the relative carbon allocation to leaf structural components increases with tree height and may also help protect the leaf blade against collapse after severe dehydration or other physical stress in canopy condition[12, 29, 38, 48]. The significantly positive relation between the C/N ratio and leaf hardness (LH) supports this assumption ($C/N = 7.96LH + 24.84$, $n = 48$, $r^2 = 0.15$, $p < 0.001$).

3. Changes in leaf photosynthetic capacities with tree height

Leaf potential photosynthetic capacities, both explained in leaf-area-based and leaf-mass-based terms, varied significantly with tree height and were limited in the upper canopy condition. The leaf-area-based photosynthetic rate at light saturation ($A_{\text{max-area}}$) showed a single-peaked pattern with tree height (Fig. 4A). The $A_{\text{max-area}}$ significantly increased with tree height from the understory up to 10 m from the ground, and then decreased linearly with tree height above 10 m, even in LMA and palisade layer thickness, which usually had positive relation with $A_{\text{max-area}}$[31, 41], still increased with tree height toward the upper canopy. This unimodal change in $A_{\text{max-area}}$ with tree height was also reported in tall conifers and temperate deciduous trees[28, 47, 67], though many tropical rainforest tree species showed simple increments of $A_{\text{max-area}}$ with tree height[5, 31, 55]. A similar value of $A_{\text{max-area}}$ was also reported by the previous study[24] for Cambodian seasonal evergreen forest; $A_{\text{max-area}}$ on the canopy, mid-story and understory trees was approximately 4.9-6.8, 4.0, and 0.9 (μmol m^{-2} s^{-1}), respectively. Conversely, the leaf-mass-based photosynthetic rate ($A_{\text{max-mass}}$) consistently decreased through the height gradient (Fig. 4B). This linear reduction on $A_{\text{max-mass}}$ was usually reported among tall temperate tree species[28, 40, 56, 66], though there is little evidence of any negative effect on tree height of $A_{\text{max-mass}}$ among tropical rainforest tree species[5, 31, 55]. These negative effects on tree height in terms of leaf photosynthetic capacities may relate to a more stressful canopy environment in a tropical dry forest compared with tropical rainforest. Further studies on the combined effects of leaf hydraulic traits such as changes in leaf water potential and whole-tree functional traits (such as rooting depth and water storage) will provide a fuller understanding of the mechanisms of drought tolerance and/or hydraulic constraints on photosynthesis in the Cambodian tropical forest trees.

4. Leaf photosynthetic traits in relation to leaf morphology, nitrogen and stomatal behavior

Differences in photosynthetic capacities with tree height are closely related to leaf nitrogen contents, as is particularly explained by the leaf mass base. It is well known that leaf-area-based photosynthetic capacities are strongly affected by various leaf morphological traits, such as leaf palisade layer thickness[31], LMA[31, 44], and leaf blade thickness[44]. However, our results clearly showed that these leaf morphological traits had no positive effect on the photosynthetic capacities (Table 1). The decrease of CO_2 diffusion conductance from the intercellular air space to carboxylation sites in the chloroplasts resulting from these thicker canopy leaves may suppress the photosynthetic rate under canopy conditions[48, 65], because $A_{\text{max-mass}}$ was negatively correlated with leaf thickness and LMA (Table 1). In contrast, there were similar changing patterns in terms of both leaf nitrogen content per unit leaf area (N_{area}) and per unit leaf mass (N_{mass}) with photosynthetic capacities (Figs. 5A, B). The N_{area} was positively correlated with tree height until 10 m from the ground, whereupon N_{area} maintained a stable value with tree height above 10 m (Fig. 5A). Leaf $A_{\text{max-area}}$ also peaked approximately 10m from the ground and then decreased toward the upper canopy (Fig. 4A). Moreover, linear reduction on N_{mass} with tree height was consistent with the linear reduction of $A_{\text{max-mass}}$ with tree height (Figs. 4B, 5B). The tree age- and/or the height- dependent decline of leaf nitrogen content usually induces a reduction in photosynthetic capacities among tall trees in temperate forests[1, 3, 47, 48, 66]. This is because nitrogen is an essential resource for photosynthetic systems as an enzyme (Rubisco) and is usually positively related to photosynthetic capacities in various plant species[15]. We also found a positive relation between leaf photosynthetic capacities and leaf nitrogen, except for that between $A_{\text{max-area}}$ and N_{area} at height exceeding 10 m from the ground (Figs. 6A,

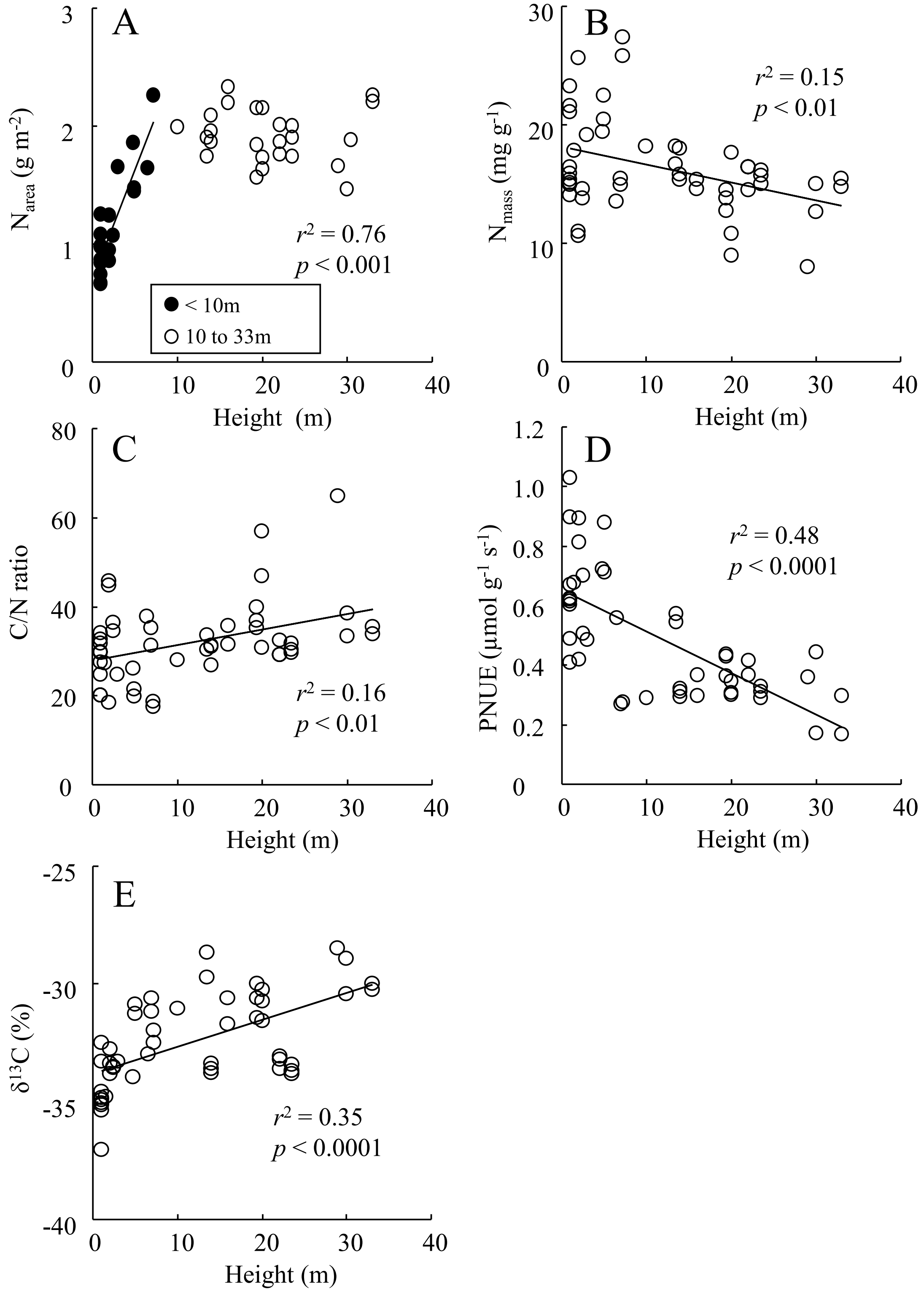

Fig. 5. Relations between the tree height and: leaf-area-based nitrogen content (N_{area}; A), leaf-mass-based nitrogen content (N_{mass}; B), leaf C/N ratio (C), photosynthetic nitrogen use efficiency (PNUE; D) and leaf stable carbon isotope composition ($\delta^{13}C$; E)

The regression lines are: A, y = 0.70 + 0.19x. B, y = 18.16 - 0.15x. C, y = 27.79 + 0.36x. D, y = 0.65 -0.01x. E, y = -33.83 + 0.12x.

B), which was consistent with previous reports[15, 30, 48]. In addition, the photosynthetic nitrogen use efficiency (PNUE, $A_{\text{max-area}}/N_{\text{area}}$) was limited in upper canopy trees (Fig. 5D), because a decrease of leaf nitrogen content is usually associated with a reduction of the photosynthetic rate[48, 54]. Many tall tree species, mostly coniferous, showed a similar reduction of PNUE and N_{mass} with tree height due to reduction of the photosynthetic rate[3, 11, 47, 57, 66, 72].

Stomatal limitation on photosynthesis also occurred at the upper canopy condition in the Cambodian tropical dry forest. To prevent water loss by transpiration activi-

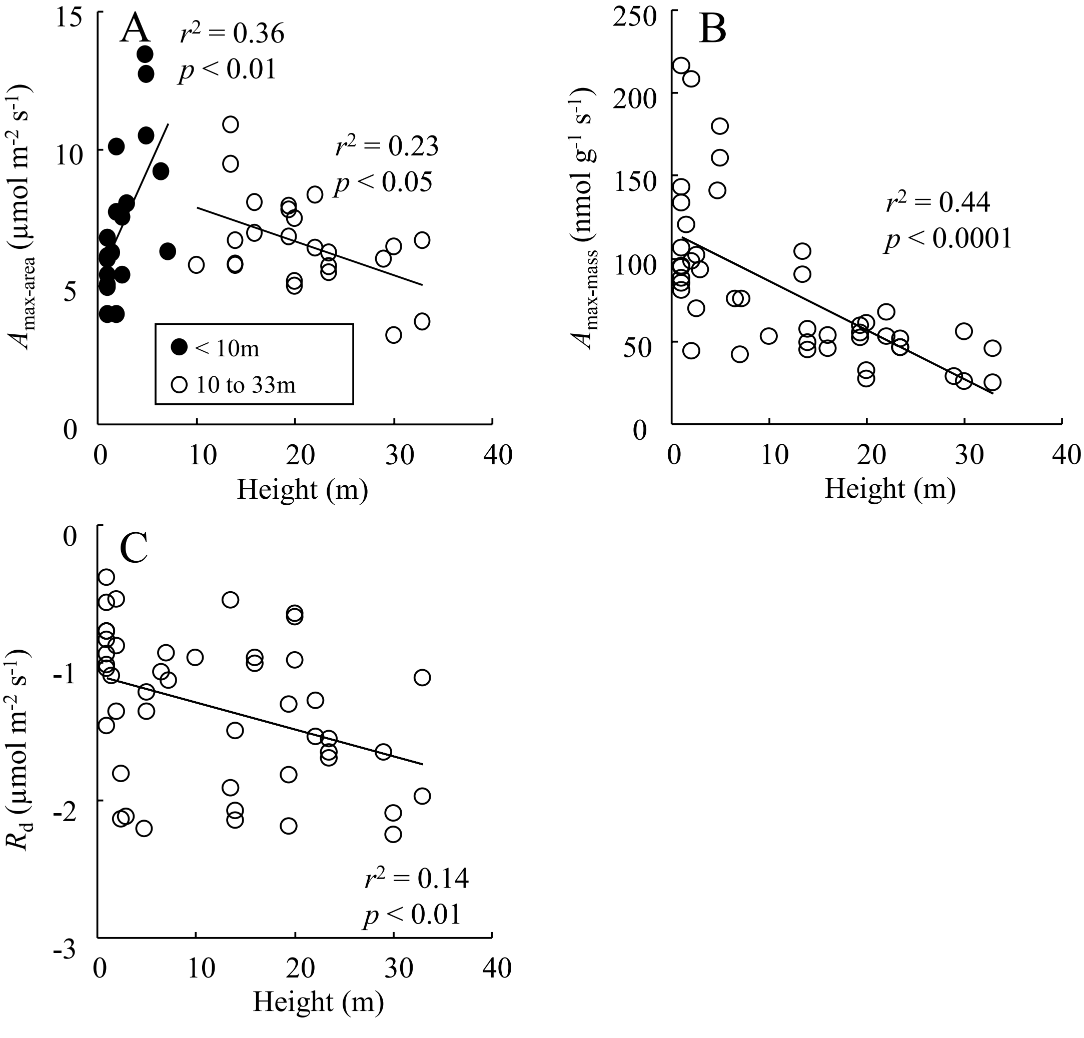

Fig. 4. Relations between the tree height and: leaf-area-based light-saturated photosynthetic rate ($A_{\text{max-area}}$; A), leaf-mass-based light-saturated photosynthetic rate ($A_{\text{max-mass}}$; B) and dark respiration rate (R_d; C)

The regression lines are: A, y = 5.24 + 0.79x; y = 9.13 – 0.12x. B, y = 115.98 – 2.96x. C, y = -1.09 - 0.02x.

Table 1. Linear regression coefficients for photosynthetic capacities versus morphological traits

	$A_{\text{max-area}}$ (µmol m^{-2} s^{-1})			$A_{\text{max-mass}}$ (nmol g^{-1} s^{-1})		
	Intercept	Slope	r^2	Intercept	Slope	r^2
LMA (g m^{-2})	7.31	-0.005	0.01	169.41	-0.872	0.58*
Stomatal density (number of stomata mm^{-2})	0.228	0.001	0.01	107.94	-0.061	0.07
Leaf thickness (µm)	8.209	-0.006	0.04	181.65	-0.447	0.45*
Palisade layer thickness (µm)	7.202	-0.005	0.01	141.01	-0.775	0.33*

An asterisk symbol (*) indicates statistically-significant differences ($P < 0.01$).

ty, leaf stomata tend to close in many plant species and this behavior became a limiting factor for leaf gas exchange under stressful conditions such as the canopy[48]. Stomatal conductance in upper canopy trees showed a relatively smaller value than that of understory or midstory trees in this study (Figs. 7A, B). In addition, a strong positive relation between both leaf-mass-based and area-based stomatal conductance and the photosynthetic rate (Figs. 6C, D) implied that stomatal behavior was dominantly related to leaf photosynthetic capacities. Ishida et al. (2006) reported a similar stomatal limitation on photosynthesis in canopy trees of tropical dry evergreen forest in Thailand. A linear increase in leaf δ^{13}C values with tree height (Fig. 5E) may imply that relative stomatal limitation of photosynthesis occurred under the canopy condition[22, 23, 46, 69]. Overall, increasing tree height results in both nitrogen and stomatal constraints on leaf photosynthetic capacity in the Cambodian tropical dry forest.

Conversely, reduction of stomatal conductance with tree height also helps maintain smaller transpiration consumption and higher leaf water use efficiency (*WUE*) in the canopy environment (Fig. 7). Increasing leaf-mass-based *WUE* with tree height was key to advantageous photosynthetic production under the drought condition of the canopy[34, 42]. In addition, linear increase in leaf δ^{13}C values with tree height (Fig. 5E) may indicate that *WUE* improved under the canopy condition by controlling the stomatal conductance[19, 23, 46, 63, 64], because δ^{13}C values are significantly related to instantaneous *WUE* and $gs_{\max}$ ($\delta^{13}C = 0.42WUE_{\mathrm{mass}} - 34.74$, n = 46, $r^2 = 0.43$, $p < 0.001$, $\delta^{13}C = -0.74gs_{\max} - 31.09$, n = 46, $r^2 = 0.25$, $p < 0.001$). However it is also noted that index of δ^{13}C for long-term *WUE* may include possible error due to variation of the

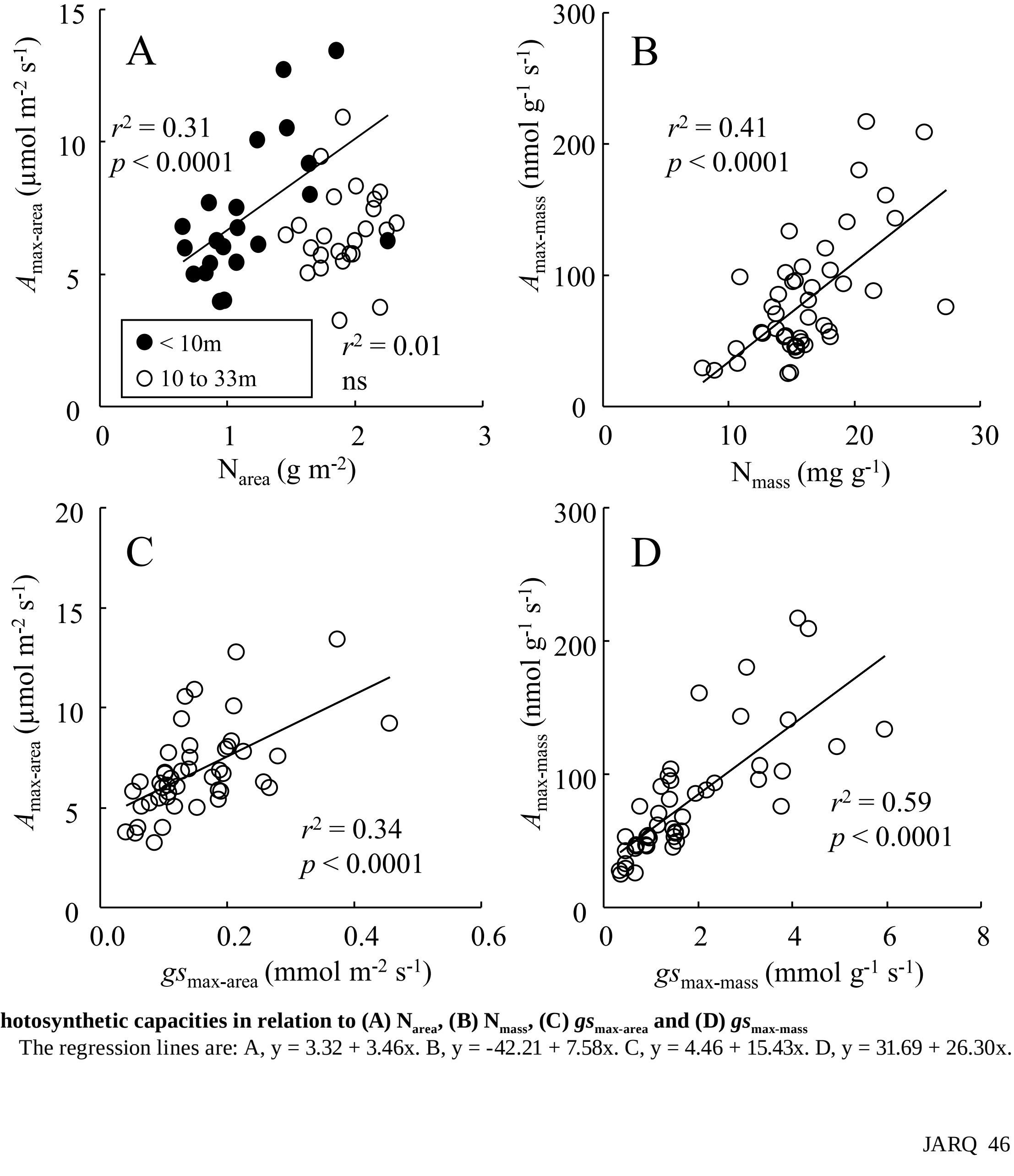

Fig. 6. Photosynthetic capacities in relation to (A) N_{area}, (B) N_{mass}, (C) $gs_{\text{max-area}}$ and (D) $gs_{\text{max-mass}}$
The regression lines are: A, y = 3.32 + 3.46x. B, y = -42.21 + 7.58x. C, y = 4.46 + 15.43x. D, y = 31.69 + 26.30x.

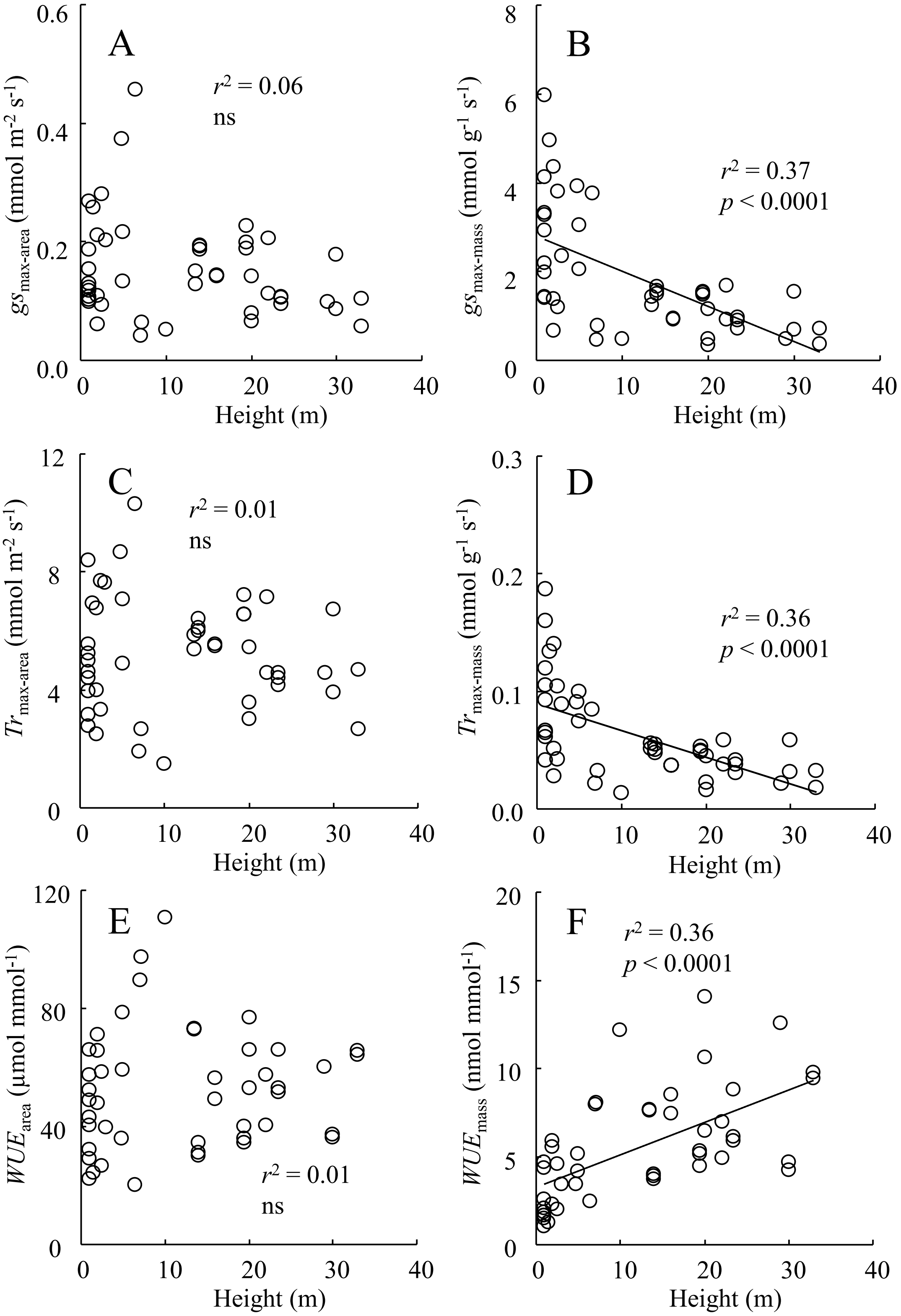

Fig. 7. Relations between the tree height and: leaf-area-based stomatal conductance ($gs_{\text{max-area}}$; A), leaf-mass-based stomatal conductance ($gs_{\text{max-mass}}$; B), leaf-area-based transpiration rate ($Tr_{\text{max-area}}$; C), leaf-mass-based transpiration rate ($Tr_{\text{max-mass}}$; D), leaf-area-based water use efficiency (WUE_{area}; E) and leaf-mass-based water use efficiency (WUE_{mass}; F)

The regression lines are: A, $y = 0.176 - 0.002x$. B, $y = 2.81 - 0.08x$. C, $y = 5.41 - 0.02x$. D, $y = 0.089 – 0.002x$. E, $y = 50.10 + 0.18x$. F, $y = 3.25 + 0.18x$.

leaf internal CO_2 concentration (C_i) from different light conditions between the measurement points in this study[17, 18, 22]. Leaf $\delta^{13}C$ in this forest trees showed similar values to many tropical rainforest tree species[2, 11, 45], though these were significantly lower than temperate and semi-arid tree species, even in canopy leaves[22, 63, 64]. This relatively lower leaf $\delta^{13}C$ under a canopy condition in the study forest may indicate that stomatal limitation caused by drought stress is weaker than the temperate and/or semi-arid forest environment.

Conclusion

This study revealed that many leaf morphological traits could be summarized in a simple and significant relation with tree height, even when many different tree species were included. In particular, the linear increment of LMA, cuticle thickness and leaf hardness contribute to higher drought tolerance in the upper canopy condition. In contrast, increasing tree height also leads to both nitrogen and stomatal constraints on leaf photosynthetic capacities at the upper canopy, though lower stomatal conductance helps reduce transpiration and maintain high *WUE* under such condition. These results suggest that leaf photosynthetic capacity and drought tolerance depend on tree height, which relate to micro-climatic conditions, and that these functions were achieved by adjusting leaf morphological and biochemical properties. The vertical variations in leaf traits associated with photosynthesis could be used to establish accurate estimation models for the CO_2 fixation and biomass production and a fuller understanding of the importance of the function of carbon pools in the forest may help conserve the Cambodian tropical dry evergreen forest[7, 13, 43].

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